SEQUENCE LISTING

<110> Reiter, Robert E.
Witte, Owen N.

<120> PSCA: PROSTATE STEM CELL ANTIGEN

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Please amend the subject application as follows:

In the claims:

Please cancel claim 48 without prejudice to pursue the subject matter of these claims in a related application. Please enter new claim 49.

--49. (new) A method for selectively killing a cell expressing Prostate Stem Cell Antigen (PSCA) comprising reacting a monoclonal antibody designated ATCC No. HB-12612, ATCC No. HB-12616, ATCC No. HB12618, or ATCC No. HB-12617 conjugated to a therapeutic agent with the cell so that the therapeutic agent so conjugated can kill the cell expressing PSCA.--

In the Specification:

At page 4, lines 21-22, please delete "FIG. 1. Nucleotide (A) and translated amino acid (B) sequences of a cDNA encoding human PSCA (ATCC Designation 209612)" and insert

--**FIG. 1A.** Nucleotide sequences of a cDNA encoding human PSCA (ATCC Designation 209612).

Fig. 1B. Translated amino acid sequences of a cDNA encoding human PSCA (ATCC Designation 209612).--

At page 5, lines 3-11, please delete "FIG. 7. Restricted Expression of PSCA mRNA in normal and cancerous tissues. **A**: RT-PCR analysis of PSCA expression in normal human tissues demonstrating high expression in prostate, placenta, and tonsils. 1ng of reverse-transcribed first strand cDNA (Clontech, Palo Alto, CA) from the indicated tissues was

amplified with PSCA gene specific primers. Data shown are from 30 cycles of amplification. **B**: RT-PCR analysis of PSCA expression demonstrating high level in prostate cancer xenografts and normal tissue. 5ng of reverse-transcribed cDNA from the indicated tissues was amplified with PSCA gene specific primers. Amplification with β-actin gene specific primers demonstrate normalization of the first strand cDNA of the various samples. Data shown are from 25 cycles of amplification. AD, androgen-dependent; AI, androgen-independent; IT, intratibial xenograft; C.L., cell line" and insert

--**FIG. 7A.** Restricted Expression of PSCA mRNA in normal and cancerous tissues. RT-PCR analysis of PSCA expression in normal human tissues demonstrating high expression in prostate, placenta, and tonsils. 1ng of reverse-transcribed first strand cDNA (Clontech, Palo Alto, CA) from the indicated tissues was amplified with PSCA gene specific primers. Data shown are from 30 cycles of amplification.

D3

FIG. 7B. Restricted Expression of PSCA mRNA in normal and cancerous tissues. RT-PCR analysis of PSCA expression demonstrating high level in prostate cancer xenografts and normal tissue. 5ng of reverse-transcribed cDNA from the indicated tissues was amplified with PSCA gene specific primers. Amplification with β-actin gene specific primers demonstrate normalization of the first strand cDNA of the various samples. Data shown are from 25 cycles of amplification. AD, androgen-dependent; AI, androgen-independent; IT, intratibial xenograft; C.L., cell line.--

At page 5, line 13, please delete "FIG. 8. Schematic representation of human PSCA, murine PSCA, and human Thy-1/Ly-6 gene structures" and insert

--**FIG. 8A.** Schematic representation of human Thy-1/Ly-6 gene structures.

D4

FIG. 8B. Schematic representation of murine PSCA gene structure.

D

54

D4 Cont'd

FIG. 8C. Schematic representation of human PSCA gene structure.--

At page 5, lines 15-19, please delete "FIG. 9. Northern blot analysis of PSCA expression. A: Total RNA from normal prostate and LAPC-4 androgen dependent (AD) and independent (AI) prostate cancer xenografts were analyzed using PSCA or PSA specific probes. Equivalent RNA loading and RNA integrity were demonstrated separately by ethidium staining for 18S and 28S RNA. B: Human multiple tissue Northern blot analysis of PSCA. The filter was obtained from Clontech (Palo Alto, CA) and contains 2ug of polyA RNA in each lane" and insert

--**FIG. 9A**. Northern blot analysis of PSCA expression. Total RNA from normal prostate and LAPC-4 androgen dependent (AD) and independent (AI) prostate cancer xenografts were analyzed using PSCA or PSA specific probes. Equivalent RNA loading and RNA integrity were demonstrated separately by ethidium staining for 18S and 28S RNA.

D5

FIG. 9B. Northern blot analysis of PSCA expression. Human multiple tissue Northern blot analysis of PSCA. The filter was obtained from Clontech (Palo Alto, CA) and contains 2ug of polyA RNA in each lane.--

At page 5, lines 21-27, please delete "FIG. 10. Northern blot comparison of PSCA, PSMA, and PSA expression in prostate cancer xenografts and tumor cell lines. PSCA and PSMA demonstrate high level prostate cancer specific gene expression. 10 μg of total RNA from the indicated tissues were size fractionated on an agarose/formaldehyde gel, transferred to nitrocellulose, and hybridized sequentially with ^{32}P-labelled probes representing PSCA, PSMA, and PSA cDNA fragments. Shown are 4 hour and 72 hour autoradiographic exposures of the membrane and the ethidium bromide gel demonstrating equivalent loading of samples. BPH, benign prostatic hyperplasia; AD, androgen-dependent; AI, androgen-independent; IT, intratibial xenograft; C.L., cell line" and insert

D6

FIG. 10-1. Northern blot analysis of PSCA expression in prostate cancer xenografts and tumor cell lines. PSCA demonstrates high level prostate cancer specific gene expression. 10 μg of total RNA from the indicated tissues were size fractionated on an agarose/formaldehyde gel, transferred to nitrocellulose, and hybridized sequentially with ^{32}P-labelled probes representing PSCA cDNA fragments. Shown are 4 hour and 72 hour autoradiogrphic exposures of the membrane. BPH, benign prostatic hyperplasia; AD, androgen-dependent; AI, androgen-independent; IT, intratibial xenograft; C.L., cell line.

FIG. 10-2. Northern blot analysis of PSM expression in prostate cancer xenografts and tumor cell lines. PSM demonstrates high level prostate cancer specific gene expression. 10 μg of total RNA from the indicated tissues were size fractionated on an agarose/formaldehyde gel, transferred to nitrocellulose, and hybridized sequentially with ^{32}P-labelled probes representing PSM cDNA fragments. Shown are 4 hour and 72 hour autoradiogrphic exposures of the membrane. BPH, benign prostatic hyperplasia; AD, androgen-dependent; AI, androgen-independent; IT, intratibial xenograft; C.L., cell line.

FIG. 10-3. Northern blot analysis of PSA expression in prostate cancer xenografts and tumor cell lines. 10 μg of total RNA from the indicated tissues were size fractionated on an agarose/formaldehyde gel, transferred to nitrocellulose, and hybridized sequentially with ^{32}P-labelled probes representing PSA cDNA fragments. Shown are 4 hour and 72 hour autoradiogrphic exposures of the membrane and the ethidium bromide gel demonstrating equivalent loading of samples. BPH, benign prostatic hyperplasia; AD, androgen-dependent; AI, androgen-independent; IT, intratibial xenograft; C.L., cell line.

At page 5, lines 29-35, please delete "FIG. 11. In situ hybridization with antisense riboprobe for human PSCA on normal and malignant prostate specimens. A: PSCA is expressed by a subset of basal cells within the basal cell epithelium (black arrows), but

not by the terminally differentiated secretory cells lining the prostatic ducts (400X magnification). B: PSCA is expressed strongly by a high grade prostatic intraepithelial neoplasia (PIN) (black arrow) and by invasive prostate cancer glands (yellow arrows), but is not detectable in normal epithelium (green arrow) at 40X magnification. C: Strong expression of PSCA in a case of high grade carcinoma (200X magnification)" and insert

--**FIG. 11A.** In situ hybridization with antisense riboprobe for human PSCA on normal prostate specimens. PSCA is expressed by a subset of basal cells within the basal cell epithelium, but not by the terminally differentiated secretory cells lining the prostatic ducts (400X magnification).

FIG. 11B. In situ hybridization with antisense riboprobe for human PSCA on normal and malignant prostate specimens. PSCA is expressed strongly by a high grade prostatic intraepithelial neoplasia (PIN) and by invasive prostate cancer glands, but is not detectable in normal epithelium at 40X magnification.

FIG. 11C. In situ hybridization with antisense riboprobe for human PSCA on malignant prostate specimens. Strong expression of PSCA in a case of high grade carcinoma (200X magnification).--

At page 6, lines 1-9, please delete "FIG. 12. Biochemical analysis of PSCA. A: PSCA was immunoprecipitated from 293T cells transiently transfected with a PSCA construct and then digested with either N-glycosidase F or O-glycosidase, as described in Materials and Methods. B: PSCA was immunoprecipitated from 293T transfected cells, as well as from conditioned media of these cells . Cell-associated PSCA migrates higher than secreted or shed PSCA on a 15% polyacrylamide gel. **C:**FACS analysis of mock-transfected 293T cells, PSCA-transfected 293T cells and LAPC-4 prostate cancer xenograft cells using an affinity purified polyclonal anti-PSCA antibody. Cells were not permeabilized in order to detect only surface expression. The y axis represents relative

cell number and the x axis represents fluorescent staining intensity on a logarithmic scale" and insert

--**FIG. 12A.** Biochemical analysis of PSCA. PSCA was immunoprecipitated from 293T cells transiently transfected with a PSCA construct and then digested with either N-glycosidase F or O-glycosidase, as described in Materials and Methods.

FIG. 12B: Biochemical analysis of PSCA. PSCA was immunoprecipitated from 293T transfected cells, as well as from conditioned media of these cells . Cell-associated PSCA migrates higher than secreted or shed PSCA on a 15% polyacrylamide gel.

FIG 12C. Biochemical analysis of PSCA. FACS analysis of mock-transfected 293T cells, PSCA-transfected 293T cells and LAPC-4 prostate cancer xenograft cells using an affinity purified polyclonal anti-PSCA antibody. Cells were not permeabilized in order to detect only surface expression. The y axis represents relative cell number and the x axis represents fluorescent staining intensity on a logarithmic scale.--

At page 6, lines 19-22, please delete "FIG. 14. Flow Cytometric analysis of cell surface PSCA expression on prostate cancer xenograft (LAPC-9), prostate cancer cell line (LAPC-4) and normal prostate epithelial cells (PreC) using anti-PSCA monoclonal antibodies 1G8 (green) and 3E6 (red), mouse anti-PSCA polyclonal serum (blue), or control secondary antibody (black). See Example 5 for details." and insert

--**FIG. 14A.** Flow Cytometric analysis of cell surface PSCA expression on prostate cancer xenograft (LAPC-9) using anti-PSCA monoclonal antibodies 1G8 and 3E6, mouse anti-PSCA polyclonal serum, or control secondary antibody. See Example 5 for details.

FIG. 14B. Flow Cytometric analysis of cell surface PSCA expression on prostate cancer cell line (LAPC-4) using anti-PSCA monoclonal antibodies 1G8 and 3E6, mouse anti-PSCA polyclonal serum, or control secondary antibody. See Example 5 for details.

FIG 14C. Flow Cytometric analysis of cell surface PSCA expression on normal prostate epithelial cells (PreC) using anti-PSCA monoclonal antibodies 1G8 and 3E6, mouse anti-PSCA polyclonal serum, or control secondary antibody. See Example 5 for details.

At page 6, line 24, please delete "FIG. 15. Epitope mapping of anti-PSCA monoclonal antibodies 1G8 and 3E6. See Example 5 for details." and insert

~~FIG. 15A. An epitope map for each of the seven disclosed antibodies.~~

FIG 15A Epitope mapping of anti-PSCA monoclonal antibodies conducted by Western blot analysis of GST-PSCA fusion proteins.

REMARKS

Claims 44-48 were pending. Claim 48 has been cancelled herein. New claim 49 is submitted herein.

New claim 49 is supported in the specification at page 12, lines 14-16; page 13, lines 1-7; page 13, lines 33-36; page 14, lines 1-18; page 23, lines 20-25; page 23, lines 29-35; and page 24, lines 1-11.

New claim 49 is supported by the specification as originally filed and do not involve new matter. Accordingly, applicants respectfully request entry of claim 49.

The changes to the specification, at pages 4-6, are to provide separate descriptions of the figures that contain multiple sub-figures. The changes do not involve new matter. Entry of these changes is requested.